II. Patentability Arguments

A. The Rejection Under 35 U.S.C. § 112, second paragraph, Should Be Witdrawn

The Examiner has rejected claim 44 under 35 U.S.C. § 112, second paragraph, alleging that there is insufficient antecedent basis for "said bacteriophages associated displayed member."

While the Applicants respectfully submit that it is clear that the associated member referred to is the binding pair member displayed on the surface of the phage, the Applicants have amended the claims to delete the word "associated" from the phrase referred to by the Examiner.

B. The Rejection Under 35 U.S.C. § 102(b), second paragraph, Should Be Withdrawn

The Examiner has rejected claims 44 and 45 as allegedly being anticipated by Smith, *et al.*, *Science*, 228:1315-1317 (June 14, 1985); Parmley, et al, *Gene*, 73:315-318, (1988); and Ladner, *et al.*, U.S. Patent No. 5,223,409. The Applicants respectfully traverse the rejections and request reconsideration in view of the present amendments and remarks for the purposes of economy, the 102(b) references will be addressed together.

Smith, *et al.*

The Examiner stated that Smith *et al.* teaches throughout the publication phage displaying antigens which are screened for specific binding antibodies (abstract).

- teaches inserting a nucleic acid encoding for (sic) an antigen (such as a fragment of a endonuclease which antigen reads on the "one member of specific binding pair (claim 44) because the antigen binds to a specific antibody (i.e., the other member of the specific binding pair member);

- teaches using filamentous phage and fusing the antigen with the geneIII coat protein (p. 1315, cols. 1-2), which reads on the phage and the geneIII coat protein of claim 44;

- teaches host cells to grow phage particles (claims 1-5) which reads on the "recombinant host cells of claim 44;

- teaches using the genome of phage f1 (co. 1, last paragraph) which reads on the phagemid genome; and

- teaches mutating the insertion (col. 2) which reads on the limitation of claim 45.

Parmley, *et al.*

The Examiner also alleges that Parmley et al, anticipates the subject matter of claim 44 because it teaches:

- inserting a nucleic acid encoding for an antigen (such as fragments of β-gal protein which the (sic) antigen reads on one member of a specific binding pair;

- teaches using filamentous phage and fusing the antigen with the Gene III coat protein;

- teaches using recombinant host cells of claim 44;

- teaches using the genome of phage which reads on the phagemid genome; and

- teaches mutating the insertions which reads on the limitation of claim 5.

Ladner, *et al.* (U.S. Patent No. 5,223,409)

Claims 44 and 45 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Ladner, et al, which according to the Examiner:

- teaches using phage display to express binding domains;

- teaches inserting a nucleic acid encoding for a binding domain which the antigen reads on "one member of a specific binding pair;"

- teaches using host cells to grow phage particles which reads on the "recombinant host cells;"

- teaches using the genome of phage which reads on the phagemid genome;

- teaches mutating the insertions; and

- teaches displaying simple chain antibodies and the scFv of claims 46-53.

The Applicants respectfully submit that neither Smith, *et al.*, nor Parmley, *et al.*, disclose a display of a properly folded functional binding domain.

A domain is defined on page 15 of the present application. This term is also widely used and is recognized and understood by those skilled in the art to mean as follows: "[T]he fundamental unit of tertiary structure is the domain. A domain is defined as a polypeptide chain or a part of a polypeptide chain that can independently fold into a stable tertiary structure." Domains are also units of function. *(See,* Introduction to Protein Structure, Carl Branden and John Tooze, Garland Publishing, Inc., New York and London, 1991) (attached hereto as Exhibit A).

Parmley *et al.*, discusses display of a 335bp fragment of beta-galactosidase corresponding to nucleotides 861-1195 in the gene sequence. This fragment of a gene encodes 112 amino acids of a much larger 380 amino acid domain. Thus, an incomplete domain is displayed, and that which is displayed has none of the functions of beta-galactosidase. It is unknown whether the three-dimensional structure of this 112 amino acid insert is the same as in the full domain, although it would be surprising if it were since interactions with other parts of the domain would be expected to affect folding and hence structure. For recognition by the rabbit polyclonal anti-beta galactosidase antibody used by Parmley *et al.* in their 'biopanning' procedure, no folding of the 112 amino acid insert would have been required since it would be expected that the polyclonal antiserum would contain antibodies against linear amino acid epitopes which could be recognised in either denatured or folded beta-galactosidase.

In support of this position, the Examiner's attention is drawn to page 25 of the present application (as published), lines 16-19, which cites Stanfied *et al., Science* 248:712-719 (1990) (attached hereto as Exhibit B) where it is pointed out that "[P]eptides can adopt a range of structures which can be different when in free solution, than when bound to, for example, an antibody, or when forming part of a protein." Stanfield *et al,* shows that a 19 amino acid peptide derived from myohemerythrin has no regular structure in the amino-terminal region in solution but forms a type II beta-turn in that

region when bound to an antibody derived by immunization with the peptide. The same 19 amino acid sequence appears to form an alpha-helix in the native myohemerythrin protein and the 19 amino acid sequence is recognised much more strongly by the antibody in apomyohemerythrin that myohemerythrin. These results show that the mere recognition by an antibody of a protein displayed on phage does not mean that the protein is correctly folded or functional since the protein can have a different overall structure and still have peptide sequences which can be recognised by an antibody.

Thus, peptides may adopt many different conformations. For example, the presence of an antibody will exert pressure on a peptide to adopt, if it can, a conformation which allows the antibody to bind. Thus, "it is the rule rather than the exception that polyclonal antisera will recognise totally denatured, and biologically inactive, proteins on Western blots" (page 26, lines 29-32, of the application, with reference to Harlow and Lane). Antibodies, A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York (1988). See also Walter and Doolittle, in "Genetic Engineering: Principles and Methods," Vol. 5, Setlow and Hollaender eds. Plenum, New York 1983, pp61-91 - relevant excerpt enclosed (pp68-69) (attached hereto as Exhibit C).

One of the conformations that a peptide *can* adopt under the influence of an antibody is the conformation it has when it forms part of the polypeptide from which it is derived. Thus, if in the polypeptide the peptide includes an epitope to which an antibody to that polypeptide binds, then in the presence of that antibody and under its influence, the free peptide can adopt the conformation which allows the antibody to bind to it. However, in the absence of the antibody the peptide is not likely to be stable in that conformation (since it is not a unit of tertiary structure - a domain) and will likely adopt other conformations.

Thus, there is no evidence that Parmley ever displayed a properly folded functional domain of a protein. In contrast, in the present invention as claimed, whole demonstrably functional domains with intrinsic binding functions such as single chain Fv fragments and alkaline phosphatase are displayed on the surface of filamentous phage,

where it is essential that they fold their correct tertiary structure in order to be able to bind to their ligands (which ability is experimentally verified as set out in the instant specification).

Smith *et al., Science* 288:1315-1316 (1985) demonstrated the display of a 171bp Sau3A fragment of *E. coli* EcoRI endonuclease. Examination of the DNA sequence of EcoRI endonuclease (A.K. Newman *et al., J. Biol. Chem.* **2562131-2139** (1981)) indicates that this would correspond to amino acids 76-133 of the protein. The X-ray crystal structure (Y.C. Kim *et al., Science* 249:1307-1309 (1990)) indicates that this region would contain just two strands of a three strand anti-parallel beta-sheet which forms part of a larger five strand beta sheet structure. Thus an incomplete and non-functional domain would have been displayed by Smith which would not have contained the amino acid sequences which are involved in binding of the protein to DNA (residues 103-241). The fragment of EcoRI endonuclease displayed on phage is recognised in the Smith paper by a **polyclonal** antiserum which would be expected to contain antibodies which would recognise **linear** epitopes which did not need to be folded to achieve their native structure.

Again, as was the case with Parmley, *et al.*, the reference fails to demonstrate the display of a properly folded functional binding domain and thus it cannot properly anticipate the present invention.

Further, the Examiner asserts that Smith uses the genome of fl which reads on the phagemid genome. However, Smith, *et al.* simply does not teach or suggest the use of phagemids as presently claimed which different from the genome of bacteriophage as discussed in more detail below.

Further, phagemids as presently claimed differ from those of Ladner *et al.* as described below.

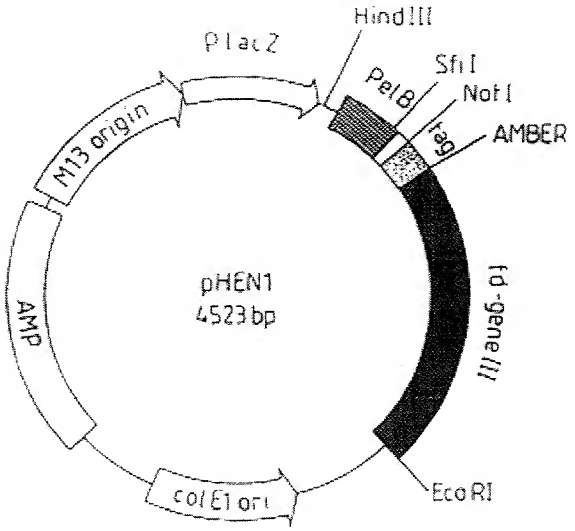
The claims as presently amended include the recitation that the only nucleotide sequences in the phagemid that are derived from filamentous bacteriophage are an origin of replication and a nucleotide sequence encoding a gene III capsid protein.

Support for this amendment can be found throughout the specification as filed. For example, paragraph [0020] of the published specification provides:

"In all embodiments where the present applicants have used phagemids, they have used a helper phage and **the only sequences derived from filamentous bacteriophage in the phagemids are the origin of replication and gene III sequences**." (emphasis added)

The Examiner is further referred to Example 24 of the specification which concerns the construction of the phagemid pHEN1 derived from pUC119. pHEN1 has the features shown in FIG. 26 which exemplifies phagemids according to the present invention.

Fig.26(a).



Because phagemids of the present invention are those in which the only filamentous bacteriophage derived nucleotide sequences are an origin of replication and a nucleotide sequence encoding a gene III protein, they differ significantly from those described in *Ladner et al.* who teach against the use of phagemids lacking a full phage genome.

The passages (column 76, lines 55-67) of Ladner *et al.* specifically points out certain distinctions between the present claimed phagemids and those of Ladner *et al.*:

"Phage prepared from these cells would be designated XY24. Phagemids such as Bluescript K/S (sold by Stratagene) are not preferred for our purposes because Bluescript does not contain the full genome of M13 and must be rescued by coinfection with competent wild-type M13. **Such coinfections** could lead to genetic recombination yielding heterogeneous phage **unsuitable for the purposes of the present invention.** Phagemids may be entirely

suitable for developing a gene that causes an IPBD to appear on the surface of phage-like genetic packages." (emphasis added)

The description of construction of phagemid vectors in Example 1 at the lines identified by the Examiner (as below) makes it clear that the phagemid vector used in the cited reference is in fact precisely of the kind that column 76 quoted above says should be used to prevent recombination, i.e. phagemids containing the full phage genome. The pGEM vectors, containing the features identified at column 106, lines 34-39, are used to assemble different gene constructs that are combined into vectors containing all of the genes of M13. See e.g. column 106, lines 54-58. The following disclosure in Ladner et al is also of relevance for the present consideration as to how Ladner et. al phagemids differ from those recited by the pending claims.

Column 106, lines 5-10:

> A. Operative cloning vectors (OCV)
> The operative cloning vectors are M13 and phagemids derived from M13 or f1. The initial construction was in the f1-based phagemid pGEM-3Zf(-)^{TM}. (Promega Corp., Madison, Wis.).

Lines 34-39:

> OCV based upon pGEM-3Zf
> pGEM-3Zf.TM. (Promega Corp., Madison, Wis.) is a plasmid-based vector containing the amp gene, bacterial origin of replication, bacteriophage f1 origin of replication, a lacZ operon containing a multiple cloning site sequence, and the T7 and SP6 polymerase binding sequences.

Lines 54-58:

> ii) OCV based upon M13mp18
> M13mp18 (YANI85) is an M13 bacteriophage-based vector (available from, inter alia, New England Biolabs, Beverly, Mass.) consisting of **the whole of the phage genome** into which has been inserted a lacZ operon containing a multiple cloning site sequence (MESS77). Two restriction enzyme sites were introduced into M13mp18 using standard methods. A BamHI recognition site (GGATCC) was introduced at the 5' end of the lacZ operon by the mutation of bases C_{6003} and G_{6004} to A and T respectively (numbering of Messing). This mutation also destroyed a unique NarI site. A SalI recognition site (GTCGAC) was introduced at the 3' end of the operon by the mutation of bases A_{6430} and C_{6432} to C and A respectively. A construct combining these variants of M13mp18 was designated M13-MB1/2. (emphasis added)

Column 111, lines 15-40:

> c) M13-gene-III-signal::bpti::mature-VIII-coat-protein
>
> We may also construct, as depicted in FIG. 5, M13-MB51 which would carry a gene encoding a fusion of M13-gene-III-signal-peptide to the previously described BPTI::mature VIII coat protein. First the BstEII site that follows the stop codons of the synthetic gene VIII is changed to an AlwNI site as follows. DNA of pGEM-MB26 is cut with BstEII and the ends filled in by use of Klenow enzyme; a blunt AlwNI linker is ligated to this DNA. This construction is called pGEM-MB26Alw. The XhoI to AlwNI fragment (approximately 300 bp) of pGEM-MB26Alw is purified. RF DNA from phage MK-BPTI (vide infra) is cut with AlwNI and XhoI and the large fragment purified. These two fragments are ligated together; the resulting construction is named M13-MB51. Because M13-MB51 contains no gene III, the phage can not form plaques. M13-MB51 can, however, render cells Km^R Infectious phage particles can be obtained by use of helper phage. As explained below, the gene III signal sequence is capable of directing (BPTI)::(mature-gene-III-protein) to the surface of phage. In M13-MB51, we have inserted DNA encoding gene VIII coat protein (50 amino acids) and three stop codons 5' to the DNA encoding the mature gene III protein.

The above passage, with reference also to Figure 5, is similarly describing the combination of a fragment of a pGEM based vector with a large fragment of MK-BPTI which is explicitly said to be "RF DNA", i.e. Replicative Form DNA which is the mature, full length (double-stranded) phage genome.

Because *Ladner et al.* discloses a method in which phagemids, unlike those of the present invention, comprise the full bacteriophage genome and further states that those which do not contain the full genome are not suitable for their purposes and further when discussing the use of helper phage they do so in the context of providing a gene III protein, the Applicants respectfully submit that *Ladner et al.* cannot properly anticipate any of the pending claims as a matter of law and, therefore, the rejections of the claims over *Ladner et al.* should be withdrawn.

B. The Rejections Under 35 U.S.C. § 103(a) over Parmley, *et al.*, and Ladner, *et al.* (WO88/06630) Should be Withdrawn

The Examiner has rejected claims 8-20 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Parmley, *et al.*, in view of Ladner, *et al.*, WO88/06630. Applicants note that claims 8-20 are currently not pending in this application. However, the Examiner refers to claims 46-49 and claims 50-53 in the discussion of the rejection and, therefore, this response is directed to those claims.

The Examiner characterizes using phage particles to display proteins of various sizes as discussed above. However, as discussed above, Parmley, does not teach the display of "binding domains" which by definition are properly folded functional polypeptides.

The Examiner has characterized Ladner as teaching the use of lambda phage to display antibody fragments such as single chain antibodies, teaching generating a large repertoire of genes encoding for single chain antibodies and displaying the antibodies on the surface of phage.

The Examiner then concludes by stating that it would have been *prima facie* obvious for one of ordinary skill in the art to generate recombinant cells comprising phage display particles displaying binding domains of antibodies or immunoglobulin or single chain antibodies.

The Applicants respectfully traverse the rejection because, *inter alia*, the present claims are directed to display of a specific binding pair member on the surface of filamentous bacteriophage while Ladner *et al.* discloses the use of bacteriophage lambda which is structurally, genomically and physiologically different from filamentous bacteriophage and, therefore, substitution of a filamentous phage as presently claimed, for a lambda phage would not, in view of these many differences have been obvious to one of ordinary skill in the art.

Among the structural differences between filamentous bacteriophage and bacteriophage lambda is that the filamentous phage has as its name suggests has a filamentous structure (attached hereto as Exhibit D). By contrast, lambda has an icosahedral capsid (20 sides) head or capsid, a collar, a tail, which tapers to a conical part which then ends in a tail fiber (attached hereto as Exhibit E). Simply stated, filamentous

bacteriophage and lambda bacteriophage are morphologically and structurally distinct with few, if any, structural features is common.

Another difference between lambda phage and filamentous phage is that the genome of lambda is double stranded DNA while the genome of filamentous phage is single stranded and is only converted into a double stranded replicative form upon entry into a host bacteria and the commencement of replication. The respective genomes of these bacteriophage are also arranged differently and comprise various different genes.

Physiologically, lambda phage and filamentous phage behave vastly differently in host cells. Lambda phage are lytic for their host cells, that is, when lambda replicates and phage particles are released from the host cells, the host cell wall is destroyed and the cell is lysed. Filamentous phage, however, are not lytic for their host cells and are assembled at and extruded from the surface of the host cell leaving the host cell intact. This has important implications with respect to the display of functional domains in that in contrast to the physiological conditions at the cell surface where filamentous bacteriophage are assembled, lambda is assembled in the cytoplasm of the host cell which is a reducing environment which can influence the ability of certain polypeptide to fold into stable functional domains.

In discussing the problem of folding in the reducing environment in the context of displaying antibody fragments, Ladner states that "one would not expect disulfide bonds to form" in the reducing environment. Ladner proposes the following solution to this problem:

> "...reduced cysteins will greatly destabilize folding of an SCA. Therefore, to get proper folding of SCAD inside a cell, one mutates the SCAD gene to change all or some of the cys's to SER, THR, ALA or Gly."

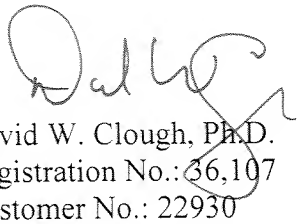
Given this proposed solution and, in view, *inter alia*, of the significant differences in structure, physiology, genome, and genome organization including the differences in sites of assembly between bacteriophage lambda and filamentous bacteriophage, the applicants respectfully submit that one of ordinary skill in the art would not be motivated to substitute bacteriophage lambda with filamentous bacteriophage and therefore Ladner cannot properly render the present invention obvious.

C. Conclusion

In view of the above amendments and remarks, Applicants respectfully submit that the instant application is in good and proper order for allowance and early notification to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite prosecution of the instant application, the Examiner is encouraged to call the undersigned at the number listed below.

Respectfully submitted,

HOWREY LLP

By: 

David W. Clough, Ph.D.
Registration No.: 36,107
Customer No.: 22930
Telephone No.: (312) 595-1408

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HOWREY LLP
ATTN: Docketing Department
2941 Fairview Park Drive, Suite 200
Falls Church, VA 22042-9922
Telephone No.: (703) 663-3600
Facsimile No.: (212) 383-7195